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Quinolizidine alkaloid status of *Acosmium* s.s., *Guianodendron* and *Leptolobium*, the segregate genera of *Acosmium* s.l.

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ABSTRACT

Extracts of leaves of five species of *Leptolobium*, the monospecific *Guianodendron* and all three species of *Acosmium* s.s. were examined for the presence of quinolizidine alkaloids by liquid chromatography–mass spectrometry and gas chromatography–mass spectrometry. The species of *Leptolobium* all contained one or more of the quinolizidine alkaloids panacosmine, lupanacosmine or isomeric forms, while leaves of *Guianodendron praeclarum* accumulated guianodendrine, a previously unreported quinolizidine alkaloid with a novel skeleton. Quinolizidine alkaloids could not be detected in leaves of any of the three species of *Acosmium* s.s., which were found to accumulate stachydrine. The quinolizidine alkaloid status of *Acosmium* s.s, *Guianodendron* and *Leptolobium* agrees with their segregation from *Acosmium* s.l. and the exclusion of *Acosmium* s.s. from the genistoid clade of legumes, as suggested by morphological characters and molecular phylogenies.

1. Introduction

The ca 17 species of Neotropical trees assigned to *Acosmium* s.l. Schott (sensu Pennington et al., 2005) have recently been divided into three genera, *Leptolobium* Vogel, *Guianodendron* Sch.Rodr. & A.M.G.Azevedo and *Acosmium* s.s., on the basis of morphological analyses (Rodrigues and Tozzi, 2006, 2007, 2008). The new monospecific genus *Guianodendron* was created to accommodate *Acosmium praeclarum* (Sandwith) Yakovlev, a species displaying vegetative and floral character sets different from other species of *Acosmium* s.l. and other papilionoid genera (Rodrigues and Tozzi, 2006). The remainder of the species in *Acosmium* s.l. formed two unrelated groups in morphology-based analyses (Rodrigues and Tozzi, 2007), requiring the reinstatement of the genus *Leptolobium* to accommodate most species (Rodrigues and Tozzi, 2008) and leaving just three species in *Acosmium* s.s. (Rodrigues and Tozzi, 2007).

The phytochemical literature lists *Acosmium* s.l. as a genus producing quinolizidine alkaloids, and this chemical character is considered an important synapomorphy for the genistoid clade of papilionoid legumes (Kite et al., 2003; Van Wyk, 2003; Wink and Mohamed, 2003). In initial phylogenetic analyses of DNA sequence data, all sampled members of *Acosmium* s.l. were those now considered to be species of *Leptolobium*, and all were placed in the genistoid clade (Pennington et al., 2001; Wojciechowski et al., 2004). However, recent molecular analyses that have sampled all three segregate genera reveal that, while *Guianodendron* and *Leptolobium* remain within the genistoid clade, *Acosmium* s.s. is excluded from it and placed in the dalbergioid clade (Cardoso et al., 2012a, 2012b, 2012c). The NE African and Madagascan genistoid genus *Dicraeopetalum* Harms was also historically classified in the broader *Acosmium* circumscription (Yakovlev, 1969), but it is reported to accumulate quinolizidine alkaloids (Asres et al., 1997; Van Wyk et al., 1993) and to be phylogenetically closer to the core genistoids (Pennington et al., 2001; Cardoso et al., 2012c).

As the quinolizidine alkaloid status of *Acosmium* s.s. and *Guianodendron* cannot be determined from the literature, with all reports of quinolizidine alkaloids from *Acosmium* s.l. being from species now assigned to *Leptolobium* (Sousa et al., 2009), we have surveyed leaf material of the three segregate genera for the presence of quinolizidine alkaloids. Prior to the review of *Sweetia* and *Acosmium* by Yakovlev (1969), many species of *Acosmium* s.l. were included in *Sweetia* Spreng. s.l., giving rise to reports of quinolizidine alkaloids in this genus in the older phytochemical literature (Balandrin and Kinghorn, 1981). Thus we also examined *Sweetia* s.s., now reduced to just *Sweetia fruticosa* Spreng. (Yakovlev, 1969), to clarify its quinolizidine alkaloid status.

2. Materials and methods

2.1. Plant material and sample extraction

Details of the taxa analysed and the sources of materials are listed in Appendix 1. Dry leaf material (20–100 mg, weighed accurately) was powdered in a pestle and mortar with sand and transferred to an Eppendorf tube. Methanol was then added (1 μl/mg of plant material) and the sample was left overnight (ca 18 h) at room temperature (ca 22 °C). Following centrifugation, the supernatant was poured into

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an autosampler vial for analysis by liquid chromatography–mass spectrometry (LC-MS) and gas chromatography–mass spectrometry (GC-MS).

2.2. Preparation of alkaloidal fraction

Following analyses of the crude extract, the sample was dried under nitrogen and dissolved in 500 μl 2 M acetic acid. An equal volume of dichloromethane was added and the mixture was vortexed in an Eppendorf tube then centrifuged to separate the phases. The upper aqueous phase was transferred to a second Eppendorff tube and ammonia solution was added to pH 11 followed by 500 μl of dichloromethane. After vortexing and centrifugation, the lower dichloromethane phase was recovered into an autosampler tube. This alkaloidal fraction was analysed by GC-MS, while the upper aqueous phase was further purified to recover very polar nitrogen-containing compounds, if appropriate. After GC-MS analysis, the alkaloidal fraction was allowed to dry and dissolved in 500 μl methanol for LC-MS analysis. The alkaloidal fractions from the two specimens of *Guianodendron praeclarum* were combined and taken to dryness, yielding a pale yellow oil (0.9 mg) comprising **3** (ca 85%) and **3a** (ca 15%).

2.3. Preparation of polar nitrogen-compound fraction

The aqueous phase (from Section 2.2) was freeze dried and dissolved in 500 μl of water, and 50 mg of Dowex 50 (H^+) 100–200 mesh ion exchange resin was added, followed by gentle mixing. After allowing the resin to settle, the liquid was discarded and the resin was washed five times with 1 ml water. A fraction containing polar nitrogen-compounds was then eluted from the resin by adding 500 μl 2 M aq. ammonia and analysed by direct injection electrospray mass spectrometry. The fractions from all the specimens of *Acosmium* s.s. examined were combined and freeze dried, affording **4** as an off-white powder (0.4 mg).

2.4. Analysis by LC-MS

Samples were analysed using a Thermo Scientific LC-MS system comprising an 'Accela' 1290 pump and autosampler interfaced to an 'LTQ-Orbitrap XL' hybrid mass spectrometer via an 'Ion-Max' electrospray source. Chromatography of 2 μl injections was performed on a 150 mm × 2.1 mm, 1.9 μm Hypersil GOLD C18 column (Thermo Scientific) using a 400 μl/min mobile phase gradient of 95:0:5 (0 min), 95:0:5 (5 min), 50:45:5 (50 min), 0:95:5 (60 min), 0:95:5 (65 min) water/methanol/acetonitrile + 1% formic acid, following 3 min pre-injection equilibration in start conditions. Tuning of the electrospray source and calibration of the mass spectrometer followed the manufacturer's procedures and recommended settings. High resolution (30,000) first order mass spectra (MS^1) were acquired in positive mode over the range m/z 125 to 2000 by the orbitrap analyser while, simultaneously, the ion trap acquired low resolution MS^1 (m/z = 125–2000) and serial mass spectra (MS^2 and MS^3) in both positive and negative modes. For serial mass spectrometry, the most abundant three or four ions in the preceding lower order scan were selected successively and fragmented using an ion isolation window of ±2 m/z units and a relative collision energy of 35%. For some analyses, the positive ion MS^2 fragments generated by the ion trap were scanned at high resolution by the orbitrap to obtain accurate mass data on the product ions.

The same system was used for direct injection electrospray mass spectrometry simply by removing the column and injecting samples into a 400 μl/min flow of 50:45:5 water/methanol/acetonitrile + 1% formic acid.

2.5. Analysis by GC-MS

Samples were analysed using an Agilent Technologies GC-MS system consisting of a 7890A gas chromatograph and a 5975C single quadrupole mass spectrometer. Chromatography of 1 μl injections vaporised at 350 °C with a 10:1 split was performed on a 30 m × 0.25 mm (i.d.), 0.25 μm DB-5 capillary column (Agilent Technologies) using 1 ml/min helium carrier gas and an oven temperature programme of either 120–350 °C or 60–250 °C, both at 5 °C/min. The mass spectrometer recorded 70 eV electron ionisation mass spectra (EIMS) over the range m/z 38–650.

2.6. Analysis by NMR spectroscopy

NMR spectra were acquired in MeOH-d_4 at 30 °C on a Bruker 400 MHz (Avance) instrument. Standard pulse sequences and parameters were used to obtain one-dimensional ^1H, and two-dimensional gradient-enhanced COSY, HSQC, and HMBC spectra. Chemical shift referencing was carried out using the internal solvent resonances at δ_H 3.31 and δ_C 49.1 (calibrated to TMS at 0.00 ppm). Stachydrine (*4*). ^1H NMR (MeOH-d_4, 30 °C): δ 4.01 (1H, dd, J = 10.4, 8.7 Hz, H-2), 3.69 (1H, m, H-5a), 3.50 (1H, m, H-5b), 3.32 (3H, s, *N*-Me), 3.15 (3H, s, *N*-Me), 2.50 (1H, m, H-3a), 2.32 (1H, m, H-3b), 2.14 (2H, m, H-4a,b); ^{13}C NMR (MeOH-d_4, 30 °C): δ 170.9 (COO^-), 77.9 (C-2), 68.2 (C-5), 52.9 (*N*-Me), 46.5 (*N*-Me), 26.6 (C-3), 20.0 (C-4).

3. Results and discussion

3.1. Leptolobium

The positive mode base ion chromatogram from the LC-MS analysis of a crude methanol extract of leaves of *Leptolobium panamense* (Benth.) Sch.Rodr. & A.M.G.Azevedo showed two prominent peaks: m/z 342.2534 at t_R 28.8 min (**1**) and m/z 358.2847 at t_R 32.4 min (**2**) (Fig. 1). The compounds responsible for these peaks did not ionise in negative mode, indicating they were basic, and were present in the alkaloidal fraction following a classical alkaloid clean-up procedure (Section 2.2.) as determined by LC-MS analysis of the fraction—they were not sufficiently volatile to be observed by GC-MS analysis. Assigning the ions to $[M + H]^+$ suggested molecular formulae of $C_{21}H_{31}N_3O$ for **1** (calculated $[M + H]^+ = m/z$ 342.2540, difference = 1.7 ppm) and $C_{22}H_{35}N_3O$ for **2** (calculated for $[M + H]^+ = m/z$ 358.2853, difference = 1.6 ppm). These formulae agree with the quinolizidine alkaloids panacosmine (for **1**) and lupanacosmine (for **2**), reported from seeds of *L. panamense* [as *Acosmium panamense* (Benth.) Yakovlev] and root bark of *Leptolobium dasycarpum* Vogel [as *Acosmium dasycarpum* (Vogel) Yakovlev], respectively (Nuzillard et al., 1999; Trevisan et al., 2008) (Fig. 2).

Panacosmine is one of only five known natural products to contain the diaza-adamantane skeleton (Buckingham, 2013), the others being acosmine, acosmine acetate and dasycarpumine (all described in the aforementioned studies of *Leptolobium*) and bowdichine from the stem bark of *Bowdichia virgilioides* Kunth, which also contains acosmine (Barbosa-Filho et al., 2004). These diaza-adamantane alkaloids are considered to be related structurally to quinolizidine alkaloids and may be derived from them (Michael, 2001); panacosmine is clearly related structurally to lupanacosmine by bridging between N-12 and C-2 and elimination of the N-12 methyl group (Fig. 2). All five species of *Leptolobium* analysed contained **1** and/or **2**, and in some species isomeric or epimeric forms were also present. Neither **1**, **2** nor isomeric forms could be detected by displaying relevant single ion chromatograms from the LC-MS analysis of crude methanol leaf extracts of *G. praeclarum* or any of the three species of *Acosmium* s.s. (Fig. 1).

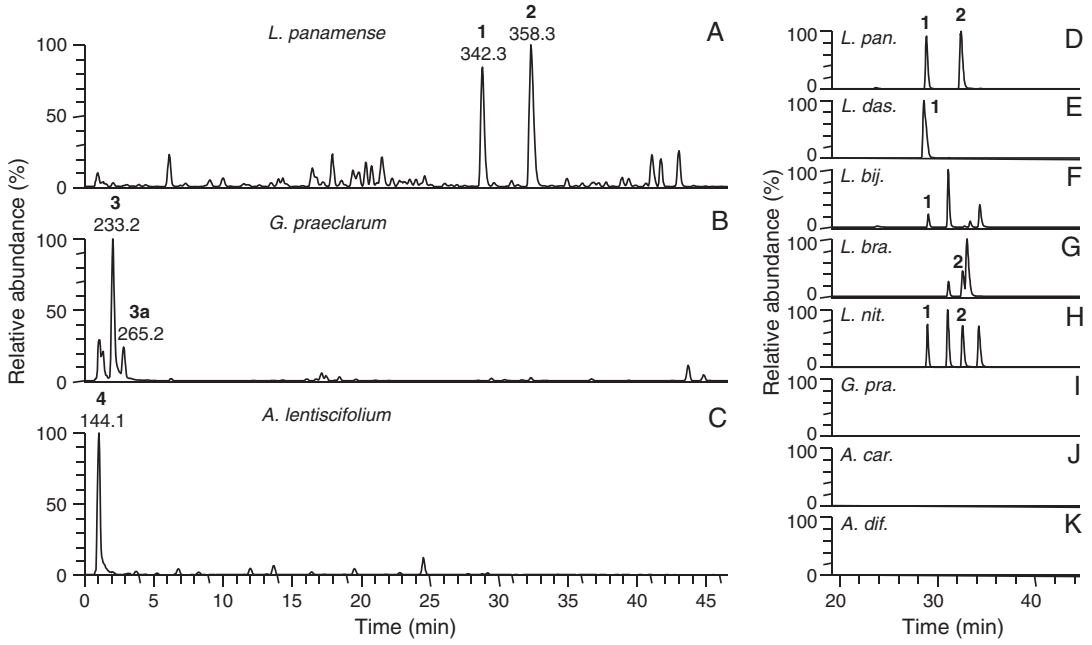

Fig. 1. Positive ion LC-MS analyses of methanol extracts of leaf material. Base ion chromatograms from analyses of *Leptolobium panamense* (A), *Guianodendron praeclarum* (B) and *Acosmium lentiscifolium*. Single ion chromatograms (*m/z* 324 and 358 combined, to reveal *1* and *2*, respectively) of *L. panamense* (D), *L. dasycarpum* (E), *L. bijugum* (F), *L. brachystachyum* (G), *L. nitens* (H), *G. praeclarum* (I), *A. cardenasii* (J) and *A. diffusissimum* (K). Unlabelled peaks in D–H are isomers of **1** or **2**.

3.2. Guianodendron

For all three specimens of *G. praeclarum* examined (including the holotype), the LC-MS base ion chromatogram of the crude methanol leaf extract showed a major peak at t_R 1.9 min due to the ion *m/z* 233.1646 (**3**) (Fig. 1). Such poor retention in the chromatographic system employed is typical for the majority of quinolizidine alkaloids (e.g. lupanine, sparteine, anagyrine), and **3** also showed alkaloidal characteristics (lack of ionisation in negative mode, alkaloidal behaviour during sample clean-up). Assuming the ion observed for **3** was the protonated molecule then the accurate mass predicts a molecular formula of $C_{14}H_{20}N_2O$ (calculated $[M + H]^+ = m/z$ 233.1648, difference = 0.8 ppm).

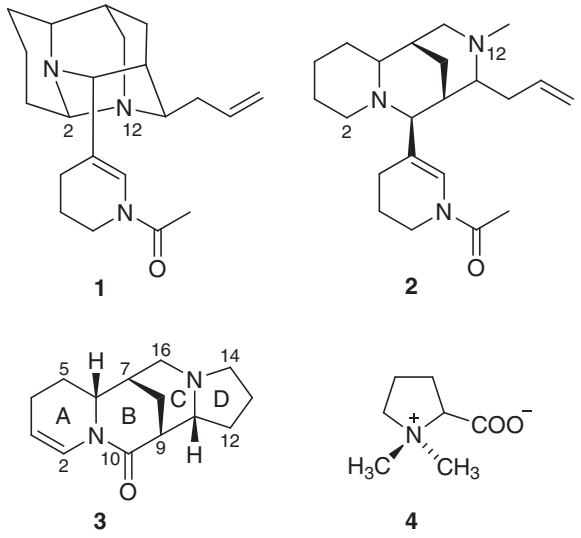

Fig. 2. Structures of the quinolizidine alkaloids panacosmine (**1**), lupanacosmine (**2**) and guianodendrine (**3**) and the betaine stachydrine (**4**) from the segregate genera of *Acosmium* s.l.

The compound could be observed by GC-MS analysis of either the crude methanol extract or the alkaloid preparation made from it, from which the following analytical data were obtained: RI (DB-5): 2249; EI-MS (70 eV), *m/z* (%): 232 (M^+, 60), 204 (15), 150 (17), 122 (100), 120 (35), 84 (39), 82 (45). These data do not match published RI and EI-MS data (Wink et al., 1995) for known quinolizidine alkaloids having the formula determined for **3** (Buckingham, 2013). The fragment at *m/z* 122, which constitutes the base ion in the EI-MS of **3**, is a feature of the EI-MS of some members of a small class of quinolizidine alkaloids characterised by fused quinolizidine and indolizidine ring systems and has been assigned as an ion containing the indolizidine ring system (Kinghorn and Balandrin, 1984). The accurate mass MS/MS spectrum of protonated **3** gave ions at *m/z* 122.0963 ($C_8H_{12}N$) and 150.0914 ($C_9H_{12}NO$), which agree with fragments with indolizidine and quinolizidine skeletons, respectively.

The structure of **3** was determined by a combination of one- and two-dimensional NMR spectroscopic techniques applied to the alkaloidal fractions from the extracts of *G. praeclarum*. A complete set of ^1H and ^{13}C resonance assignments is given in Table 1, based on correlations observed in COSY, HSQC, and HMBC spectra. The structure of this compound, to which the trivial name of guianodendrine has been given, is shown in Fig. 2. As the MS fragmentation data suggested, guianodendrine (**3**) is a member of the fused quinolizidine/indolizidine type of quinolizidine alkaloids. Uniquely however, it contains an oxo-group at C-10, in contrast to previously described fused quinolizidine/indolizidine alkaloids of the leontidine-type, which are 2-oxo derivatives (Kinghorn and Balandrin, 1984). The location of the oxo group in **3** was confirmed by the long-range correlation from H-8a (δ_H 2.21) to the lactam carbonyl at δ_C 170.8 (C-10), and the downfield shifts observed to both H-9 (δ_H 2.76) and C-9 (δ_C 40.6). The remainder of the structure was readily defined using a combination of COSY and HMBC (Table 1) data. With the exception of the 5-membered D-ring, guianodendrine (**3**) is analogous to 2,3-dehydro derivatives of the 10-oxosparteine series of quinolizidine alkaloids i.e. based on either aphylline (10-oxosparteine) or its 11-epimer, epiaphylline (10-oxo-α-isosparteine). The vicinal coupling constants for H-7_{eq} with H-16_{ax} and H-16_{eq} in **3** are small and

Table 1
^{1}H and ^{13}C NMR spectroscopic data for **3** (MeOH-d_4, 30 °C).

Atom	δ ^{13}C	δ ^{1}H (*J* in Hz)	HMBC ($^{2}J_{HC}$, $^{3}J_{HC}$)
2	124.7	7.16 *dt* (8.4, 2.0)	4
3	112.9	5.28 *ddd* (8.4, 5.0, 2.6)	4
4	23.9	2.28 *m*	2
		2.20 *m*	
5	26.8	1.94 *m* (2H)	3, 4, 6, 7
6	58.7	3.74 *m*	7, 16
7	33.0	2.24 *m*	
8	22.0	2.21 *m*	6, 9, 10
		1.99 *m*	
9	40.6	2.76 *m*	
10	170.8	–	
11	65.0	3.50 *m*	
12	25.2	2.10 *m*	9, 11, 13
		2.02 *m*	
13	21.8	2.19 *m*	11
		2.02 *m*	
14	56.2	3.08 *m*	13, 16
		2.99 *m*	11, 13, 16
16	49.1	3.24 *dt* (12.9, 2.3)	8, 11
		2.90 *dd* (12.9, 3.2)	6, 7, 8, 14

of similar magnitude (Table 1), implying a common *gauche* relationship that is indicative of a chair-conformation for the C-ring (Galasso et al., 2004). Theoretical considerations based on density functional theory analysis also suggest that the preferred conformation of 10-oxosparteines is with each of rings A–D in the chair conformation, or more precisely, with a chair–sofa A/B-quasi-*trans*, chair–chair C/D-quasi-*cis* disposition (Galasso et al., 2004). Thus the conformation of *3* is probably analogous to that of 2,3-dehydroepiaphylline (2,3-dehydro-10-oxo-α-isosparteine), which has been reported from leaf extracts of *Genista monspessulana* (L.) L.A.S.Johnson, but using the synonym *Cytisus monspessulanus* L. (Nihei et al., 2002).

A minor alkaloid (**3a**) was observed accompanying guianodendrine (**3**) in both LC-MS and GC-MS analyses. Its molecular formula was CH_3OH greater than **3** and the loss of 32 Da from $[M + H]^+$ following MS/MS to give an ion (*m/z* 233) that had the same product ion spectrum as that of protonated **3** suggested that **3a** could be a methoxy derivative of **3** generated by methanolysis of the A-ring double bond. A small amount of **3a** (ca 15%) was present in the sample of **3** analysed by NMR spectroscopy. Although the majority of its resonances were overlapped by those of guianodendrine, a singlet at δ_H 3.23 (δ_C 55.4) was assigned to an OMe group. In the HMBC spectrum, this OMe resonance correlated with a methine carbon at δ_C 80.7, which corresponded to a proton resonance at δ_H 5.84 (*br t*, $J = 2.7$ Hz) by HSQC. In the COSY spectrum, the CH(OMe) resonance at δ_H 5.84 correlated with δ_H 1.72 and 1.92. These observations are consistent with the identification of **3a** as a methoxy derivative of guianodendrine (**3**). The downfield shifts of the methine group resonances at δ_H 5.84 and δ_C 80.7 also suggest that the OMe group of **3a** is in close proximity to N-1, i.e. located at C-2 rather than C-3; however, further analysis of a purified sample is required for confirmation of structure.

In accumulating guianodendrine, the leaves of *Guianodendron* are distinct from those of *Leptolobium* species, in which guianodendrine could not be detected. This divergence in alkaloid chemistry provides further support to the segregation of these genera based on morphological and molecular data (Rodrigues and Tozzi, 2006, 2007; Cardoso et al., 2012a, 2012c). Phylogenetic analysis of combined DNA sequence and morphological data suggests that *Guianodendron* is sister to *Diplotropis* sect. *Racemosae* H.C.Lima, although morphologically distinct from members of that taxon (Cardoso et al., 2012b).[1] We could not detect guianodendrine in LC-MS analyses of leaf extracts obtained from specimens representing *Diplotropis* sect. *Racemosae*: *Diplotropis duckei* Yakovlev, *D. racemosa* (Hoehne) Amshoff var. *racemosa* or *D. racemosa* var. *rosae* H.C.Lima. The chemical data acquired from these specimens were not examined further for the present study, although the latter specimen, at least, clearly contained an accumulation of alkaloids.

3.3. Acosmium s.s.

In the LC-MS analyses of the crude methanol leaf extracts of all three species of *Acosmium* s.s., the major peak was again due to a poorly retained compound, eluting near the solvent front, and giving an ion at *m/z* 144.1026 (**4**) (Fig. 1). Assuming this ion to be $[M + H]^+$, the predicted molecular formula of $C_7H_{13}NO_2$ has insufficient carbons to construct a quinolizidine ring system. Furthermore, **4** failed to partition into dichloromethane from basified aqueous solution but could be recovered using a standard ion exchange clean-up method for polar nitrogen-compounds (Section 2.3). Application of this procedure to the combined preparations from three of the specimens studied (total dry weight of leaf material, 193 mg) yielded **4** in sufficient amounts for analysis by NMR spectroscopy (Section 2.6). The ^{1}H and ^{13}C NMR spectroscopic assignments obtained for **4** were in good agreement with those of the betaine stachydrine (1,1-dimethylpyrrolidinium-2-carboxylate), taking into account a small solvent effect (Sargenti et al., 1993; Daughtry et al., 2012). The compound was sufficiently volatile as the free base for analysis by GC-MS without the need for derivatization, giving the following data that were identical to a commercial standard of stachydrine: RI (DB-5): 1034; EI-MS (70 eV), *m/z* (%): 143 (M^+, 3), 84 (100), 82 (14), 55 (4), 42 (18).

In none of the LC-MS analyses of the three *Acosmium* species were any compounds detected that could be candidates for quinolizidine alkaloids, and the alkaloidal preparation (Section 2.2) showed no chromatographic peaks when analysed by GC-MS. Therefore, we could find no evidence that leaves of *Acosmium* s.s. accumulate quinolizidine alkaloids. Although larger quantities of leaves and material from other organs would need to be analysed to prove that the genus does not produce quinolizidine alkaloids, the status of *Acosmium* s.s. as a non-producer of quinolizidine alkaloids does concur with its phylogenetic position outside of the genistoid clade (Cardoso et al., 2012a, 2012c).

3.4. Sweetia s.s.

No candidates for quinolizidine alkaloids could be detected in the leaf extract of *S. fruticosa*, either from LC-MS analysis of the crude methanol extract, or GC-MS analysis of an alkaloid preparation from it. There are no reports in the literature of alkaloids from *S. fruticosa* and the lack of quinolizidine alkaloids agrees with the position of *Sweetia* s.s. outside of the genistoid clade—the genus being placed in the vataireoid clade in molecular phylogenetic analyses (Pennington et al., 2001; Wojciechowski et al., 2004; Cardoso et al., 2012c, 2013).

3.5. Conclusion

Some chemical characters have been taxonomically useful in legumes, particularly in the genistoid clade of the Papilionoideae (e.g. Van Wyk, 2003), and in the present work they again prove valuable in supporting the updated classification of phylogenetically unrelated genera. The quinolizidine alkaloid status of *Leptolobium*, *Guianodendron* and *Acosmium* s.s. agrees with the segregation of *Acosmium* s.l. and the exclusion of *Acosmium* s.s. from the genistoid clade.

The history of reclassification of some species in *Acosmium* s.l. has, however, created a confusing chemical nomenclature due to novel quinolizidine alkaloids being given common names based on the genus to which the taxon was assigned at the time—thus acosmine was described from *L. dasycarpum* under the synonym *A. dasycarpum* (Trevisan et al., 2008) and sweetinine was first described from

[1] The genus *Staminodianthus* D.B.O.S. Cardoso, H.C. Lima & L.P. Queiroz has recently been described to accommodate species previously placed in *Diplotropis* sect. *Racemosae*. Cardoso et al. (2013). *Phytotaxa* 110: 1–16.

Leptolobium elegans under the synonym *Sweetia elegans* (Vogel) Benth. (Balandrin and Kinghorn, 1981). Unfortunately, *Sweetia* s.s. does not contain sweetinine and *Acosmium* s.s. does not contain acosmine. Such confusion might be avoided in the future, when studying genera suspected of being very broadly circumscribed in traditional classifications, by being cautious about designating common chemical names derived from the genus name, as new data, especially from molecular analyses, may require the reinstatement of historical genus names, or publication of new ones, for more narrowly defined generic segregates.

We feel justified in naming **3** as guianodendrine from the strong morphological, molecular and chemical evidence showing that the monospecific genus *Guianodendron* is distinct from other genistoid genera and unlikely to be subjected to future nomenclatural change at the genus level. Furthermore, **3** cannot sensibly be given a derivative name based on the common name of another quinolizidine alkaloid. Our data also suggests that **3** is a major and characteristic chemical feature of the leaves of *G. praeclarum*, and so it merits taking a common name that reflects the source genus.

Appendix 1. Details of specimens studied

Taxon: Country, Date, Voucher specimen (or Kew living collections accession no.), RBG Kew phytochemical reference no.

Acosmium cardenasii H.S.Irwin & Arroyo: Brazil, 09/10/1985, *J.A. Ratter* et al. *5134* (K), BI-22691. *Acosmium diffusissimum* (Mohlenbr.) Yakovlev: Brazil, 17/11/1991, *G. Hatschbach & M. Hatschbach 55210* (K), BI-22692. *Acosmium lentiscifolium* Schott: Brazil, 02/04/1991, *G.P. Lewis & S.M.M. de Andrade 1962* (K), BI-22383. *A. lentiscifolium*: Brazil, 15/2/2007, *H.C. de Lima* et al. *6514* (K), BI-22693. *Diplotropis duckei* Yakovlev: Brazil, 24/11/1986, *H.C. de Lima* et al. *2776* (K), BI-22771. *Diplotropis racemosa* (Hoehne) Amshoff var. *racemosa*: Guyana, 06/09/1937, *N.Y. Sandwith 1393* (K), BI-22772. *Diplotropis racemosa* var. *rosae* H.C. Lima: Brazil, 22/04/1986, *C.A. Cid Ferreira* et al. *7129* (K), BI-22773. *Guianodendron praeclarum* (Sandwith) Sch.Rodr. & A.M.G.Azevedo: Guyana, 17/05/1943, *Forest Dept. of British Guiana field no. F1297* (*record no. 4033*) (K), BI-22385. *G. praeclarum*: Guyana, 17/8/1937, *N.Y. Sandwith 1109* (K), BI-22694. *G. praeclarum*: Guyana, 01/03/1934, *Forest Dept. of British Guiana field no. A69* (*record no. 2353*) (holotype, K), BI-22770. *Leptolobium bijugum* Vogel: Brazil, 12/05/1987, *H.C. de Lima* et al. *2956* (K), BI-22687. *Leptolobium brachystachyum* (Benth.) Sch.Rodr. & A.M.G.Azevedo: Brazil, 12/03/2009, *D.C. Zappi* et al. *2090* (K), BI-22686. *Leptolobium dasycarpum* Vogel: Brazil, 16/11/2009, *B.B. Klitgaard 1185*, BI-22469. *Leptolobium nitens* Vogel: Brazil, 28/03/1999, *M.A. da Silva* et al. *4203* (K), BI-22689. *Leptolobium panamense* (Benth.) Sch.Rodr. & A.M.G.Azevedo: El Salvador, 20/02/1989, *G.P. Lewis & C.E. Hughes 1739* (K), BI-22384. *Leptolobium tenuifolium* Vogel: Brazil, 15/03/2007, *R.D. Ribeiro* et al. *809* (K), BI-22690. *Sweetia fruticosa* Spreng.: Paraguay, undated, *Hassler 6211* (K), BI-22380.

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